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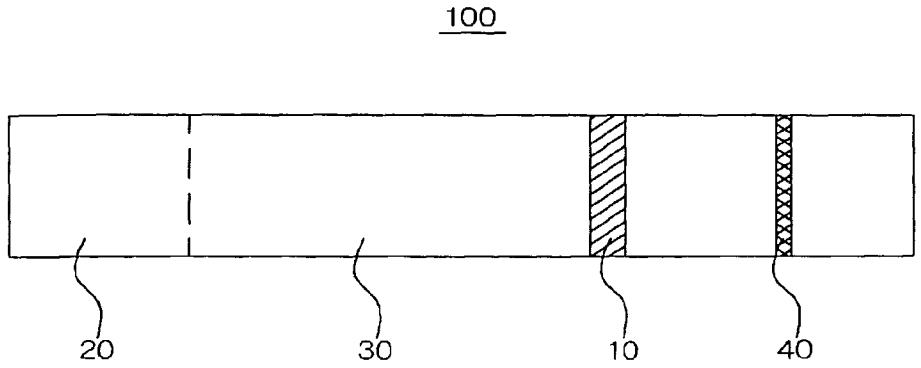

(57) Abstract: The present invention relates to a method and a kit for diagnosing and/or predicting the occurrence of cancer or the risk of contracting a cancer by measuring the concentration of a cancer screening antigen(CSA) in blood, which changes before the occurrence of the cancer in a patient. The method of diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising the steps of: determining a concentration of galectin-3 in a blood sample by reacting the blood sample with a monoclonal antibody of the galectin-3; comparing the determined concentration of the galectin-3 with concentration of the galectin-3 in a blood sample of a normal human; and predicting the risk of contracting a cancer if the determined concentration is greater than the concentration of the galectin-3 in blood of the normal human.

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METHOD AND KIT FOR PREDICTING CANCER

FIELD OF THE INVENTION

The present invention relates to a method and a kit for predicting cancer in a simple and easy manner. More particularly, the present invention relates to a method and a kit for predicting and/or diagnosing the occurrence of cancer or the risk of contracting a cancer by measuring the concentration of a cancer screening antigen (CSA) in blood, which changes before the occurrence of the cancer in a patient. The present invention is particularly useful for (1) a patient having a cancer, but no subjective symptom, (2) a patient having a benign tumor, a chronic inflammation or gastritis, but no subjective symptom, and (3) a normal person. The patients in the state of above (1) and (2) are capable of predicting the occurrence of cancer or the risk of contracting a cancer with the kit and method according to the present invention, and may have a more precise medical examination, which helps diagnosis of the cancer in early stage.

BACKGROUNDS OF THE INVENTION

The detection of a cancer in early stage is most important in healing the cancer. In most case, the subjective symptom due to a cancer is revealed after the cancer is complicated. The medical check up examination for cancer in hospital is generally not easy for ordinary person, and this makes it difficult to find out the cancer in early stage. There is a continuing need for a method to predict the occurrence of cancer or the risk of contracting a cancer in a simple and easy manner so that ordinary person can self-examine the occurrence of cancer conveniently and in early stage of the cancer development.

To develop simple and easy method to diagnose and remedy cancer, immunologists have tried to find out tumor-specific transplantation antigens (TSTA). However, due to the characteristic fact that the tumor is derived from the normal cell of a human, the TSTA is not identified except for some malignant epithelioma. Meanwhile, it is also tried to use tumor-associated transplantation antigens (TATA) such as an alpha-fetoprotein (AFP) and a carcinoembryogenic antigen (CEA), which exists in normal cell, but increases in tumor cell, for diagnosing the cancer. However, the over-manifestation of TATA occurs only after the tumor is complicated. Therefore, the method for diagnosing tumor with TATA is not much helpful in early diagnosing the cancer.

Galectins is a β-galactoside-binding lectin, and exists in most plant and animal including a poriferan, an eelworm, mammamlia, etc, and has high affinity to a laminin which is an extracellular matrix (ECM) of animal, and known to have close relation with the metastasis of tumor [26, 27]. In addition, it is also known that the galectins have close relation with the growth and transformation of a cell, and metastasis of a tumor. Until now, nine kinds of galectin are identified, and form a galectin family. The galectins have molecular weight of 14-36kDa, and exists in monomer or dimer forms. The galectin-1, galectin-3, and galectin-8 exist in various internal organs, and galectin-2, galectin-4, galectin-5 and galectin-7 exist in specific internal organs or cells [2, 10, 15, 17, 25].

Galectin-3 is also called as CBP-35, Mac-2, ε BP, RL-29, L-34, L-31 etc, and discovered by Ho and Springer [12] in the macrophage activated by

thioglycollate, and is a protein having molecular weight of about 26-32 kDa [27]. The galectin-3 relates with the growth, differentiation, malignant degeneration, and embryo formation of a cell, and also relates with the hypersensitivity reaction of a cell mediated with IgE, and plays important role in the binding between cells, and between cells and matrix. The galectin-3 accelerates the uptake of the calcium ion in human Jurkat T-cell [8], and has relation with the apoptosis of a T-cell [29]. Galectin-3 can exist in the form of ribonucleoprotein (RNP)/galectin-3 complex, and effects the pre-mRNA substrate and the RNA splicing process [7].

It is also known that the galectin-3 can be released from a cell without signal peptide [31]. As the biological materials having the same releasing property, interleukin-1 (IL-1) and fibroblast growth factor (FGF) are known, but the precise releasing mechanism is not identified yet. This releasing property is quietly different form that of general soluble protein in eukaryotic cell.

The galectin-3 protein is known to have close relation with malignant tumor, and occurs in colon cancer, skin cancer, thyroid gland cancer, breast carcinoma etc [5, 6, 9, 14, 18, 19, 20, 23, 24, 28]. There are much less research in the field of stomach cancer than colon cancer, lung cancer, breast carcinoma and malignant melanoma. The galectin-3 does not manifest in the normal liver cell, but manifests in liver cancer and cardiac cirrhosis [13]. In case of the thyroid gland cancer, it is known that the manifestation of the galectin-3 can be used as a marker before cancer operation [20]. In case of stomach cancer, it is reported that the galectin-3 does not manifest in normal stomach tissue, but manifests in the stomach cancer [30]. In summary, the heretofore researches indicate that the

manifestation of galectin-3 has close relation with the malignant tumor. However, the precise relation of galectin-3 with the tumor development, especially with stomach cancer development, is not established. Furthermore, the use of the galectin-3 in predicting and/or diagnosing the occurrence of cancer or the risk of contracting cancer is not known.

SUMMARY OF THE INVENTION

In view of the foregoing, I identified the relation of manifestation of galetin-3 and development of tumor, and discovered the over-manifestation of galetin-3 in the stage of a benign tumor or a chronic inflammation and gastritis, which is a former or an initial stage of tumor development. Thus, the present invention is directed to the simple and easy method of using the over-manifestation of galetin-3 in the former and initial stage of tumor development.

Therefore, it is an object of the present invention to provide a method and a kit for diagnosing and/or predicting the occurrence of cancer or the risk of contracting a cancer in the former and initial stage of tumor development by measuring the concentration of galectin-3 in blood, which is over-manifested in the stage of a benign tumor (adenoma) or a chronic inflammation which is the former or initial stage of the malignant tumor (carcinoma). The user recognizing the occurrence of cancer or the risk of contracting a cancer with the kit and method according to the present invention may have a more precise medical examination in hospitals, which results in the early and exact diagnosis of the cancer.

In other words, the kit and method according to the present invention may help the user to self-examine his or her risk of contracting a cancer in the initial

stage of tumor development, and contributes to the early finding of cancer, especially the stomach cancer, in the early stage of tumor development. Therefore, it is other object of the present invention to provide a method and a kit for diagnosing and/or predicting the occurrence of cancer of user having no subjective symptom.

To accomplish these and other advantages, the present invention provides a method of diagnosing and/or predicting the occurrence of a cancer or the risk of contracting a cancer comprising the steps of: determining a concentration of galectin-3 in a blood sample by reacting the blood sample with a monoclonal antibody of the galectin-3; comparing the determined concentration of the galectin-3 with concentration of the galectin-3 in a blood sample of a normal human; and predicting the risk of contracting a cancer if the determined concentration is greater than the concentration of the galectin-3 in blood of the normal human.

The present invention also provides a method of diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising the steps of: preparing an assay strip having a reaction part, a sample injection part, and a membrane for providing passage from the sample injection part to the reaction part, wherein a capture antibody of galectin-3 or galectin-3 is immobilized on the reaction part; moving a blood sample including galectin-3 and a gold-conjugated tracer antibody of the galectin-3 trough the membrane from the sample injection part to the reaction part; and predicting the risk of contracting a cancer according to the color change of the reaction part.

The present invention further provides a kit for diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising an assay strip having a reaction part, a sample injection part, and a membrane for providing sample passage from the sample injection part to the reaction part, wherein the reaction part includes a capture antibody of galectin-3 or galectin-3 immobilized thereon so that a color of the reaction part is determined according to the concentration of galectin-3 in a blood sample when the blood sample including galectin-3 and a gold-conjugated tracer antibody of the galectin-3 reaches from the sample injection part to the reaction part trough the membrane.

The present invention further provides a kit for diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising: a microplate for immobilizing galectin-3 in a blood sample; and a monoclonal antibody to react with the galectin-3 immobilized on the microplate to induce a color change of the microplate.

The present invention further provides a method of diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising the steps of: determining a cancer screening antigen which manifests in the stage of a adenoma or a chronic inflammation which are the former stages of the malignant tumor development; determining a concentration of the cancer screening antigen in a blood sample by reacting the blood sample with a monoclonal antibody of the cancer screening antigen; comparing the determined concentration of the cancer screening antigen with a concentration of the cancer screening antigen in a blood sample of a normal human; and predicting the risk of contracting a cancer if the determined concentration is substantially greater or

lesser than the concentration of the cancer screening antigen in blood of the normal human.

BRIEF DESCRIPTION OF THE DRAWINGS & PHOTOGRAPHS

A more complete appreciation of the invention, and many of the attendant advantages thereof, will be readily apparent as the same becomes better understood by reference to the following detailed description when considered in conjunction with the accompanying drawings and photographs, wherein:

Fig. 1A is a microscope photograph showing the manifestation of galectins-3 at Auerbach plexus surrounded by normal cell (magnifying ratio: 200);

Fig. 1B is a microscope photograph showing the manifestation of galectins-3 at semi-differentiated signet ring cell (magnifying ratio: 200);

Fig. 2A is a microscope photograph showing the manifestation of galectins-3 at intestinal metaplasia (magnifying ratio: 100);

Fig. 2B is a microscope photograph showing the manifestation of galectins-3 at tubular carcinoma (magnifying ratio: 200);

Fig. 3 is a microscope photograph showing the manifestation of galectins-3 at well-differentiated tumor tissue and semi-differentiated signet ring cell (magnifying ratio: 200);

Fig. 4 is a graph showing the ratio of 5 years survival according to the degree of the manifestation of galectins-3; and

Fig. 5 is a graph showing the concentration of galectins-3 in blood of normal human, patient having stomach cancer, and patient having chronic gastritis. The concentrations of galectins-3 in blood sample, which is diluted successively,

were represented by absorbance measured by the ELISA method. In Fig.5, x-axis represents dilution ratio of the blood sample, and y-axis represents absorbance at 490nm. Each mark in Fig.5 is depicted to represent mean ± standard deviation.

Fig.6 is a plan view of an assay strip forming the kit according to an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

For a better understanding of the present invention, reference will now be made in detail to the following disclosures and appended claims.

The present invention provides a method for diagnosing and/or predicting the occurrence of cancer or the risk of contracting a cancer, such as stomach cancer, liver cancer, thyroid gland cancer etc, at the former or initial stage of tumor development. Heretofore, the cancer is diagnosed by detecting the concentration of the tumor-associated transplantation antigens (TATA), which exists in normal cell, but increases in tumor cell. However, the over-manifestation of TATA occurs when the tumor is complicated, i.e. after the initial stage of the tumor development. Therefore, the method for diagnosing tumor with TATA is not much helpful in early diagnosing the cancer.

In contrast, the present invention is directed to a new concept for early diagnosing the tumor development. The method according to the present invention is to diagnose and/or predict the risk of contracting a cancer with an antigen which is over-manifested in blood before the development of the malignant tumor(carcinoma), rather than using an antigen which is over-manifested after the

development of the carcinoma. The antigen, which is used in the present invention, is defined as a cancer screening antigen (CSA).

The present invention also provide a kit utilizing the characteristics of the cancer screening antigen (CSA) so that an ordinary user can diagnose and/or predict the occurrence of a cancer such as stomach cancer, liver cancer, thyroid gland cancer etc, preferably the stomach cancer, in early stage of the cancer development and in simple and easy manner. According to the present invention, the suitable cancer screening antigen is the galectin-3. The galectin-3 is not manifested in normal tissue such as normal stomach tissue, liver tissue and thyroid gland, but over-manifested in the stage of a benign tumor (adenoma) or a chronic inflammation which is the former stage of the malignant tumor(carcinoma). Thus, the galectin-3 can be used for early diagnosing the stomach cancer. In addition, since the liver cancer and thyroid gland cancer have same characteristics with the stomach cancer, the galectin-3 can be used for early diagnosing the liver cancer and the thyroid gland cancer.

The method of using the galectin-3 in predicting the occurrence of a tumor such as stomach cancer includes the steps of obtaining a blood sample of a user, determining the concentration of the galectin-3 in the blood sample via antigen-antibody reaction, and comparing the determined concentration of the galectin-3 with the concentration of the galectin-3 in a blood of a normal human who is not in the stage of a benign tumor (adenoma), a chronic inflammation, or the malignant tumor(carcinoma). The galectin-3 is not manifested in normal blood, but over-

manifested in the stage of the adenoma or the chronic inflammation, which is the former stage of the carcinoma, and the over-manifestation is maintained in the stage of the carcinoma. Thus, if the concentration of the galectin-3 in the blood sample of a user is greater than that of the normal human, the user might be in the initial or former stage of the disease, and there is high probability of contracting the tumor.

Because the method according to the present invention diagnoses cancer by determining the concentration of the galectin-3 in blood sample rather than directly examining the tissues of stomach, liver and thyroid gland, the discrimination of the specific type of cancer, such as stomach cancer, liver cancer and thyroid gland cancer, cannot be complete. However, if the test result is positive, the user may have a more precise medical examination, which results in the early and exact diagnosis of the cancer.

The first step of diagnosing tumor according to the method of the present invention is to determine the concentration of the galectin-3 in the blood sample by reacting the blood sample with a monoclonal antibody of the galectin-3. The concentration of the galectin-3 in the blood sample can be determined by an immunological test method, preferably by an enzyme- immunological test method which uses the antigen-antibody reaction.

In the case of using an enzyme-linked Immunosorbent assay (ELISA), which is one of the enzyme- immunological test methods, the antigen (galectin-3) in a blood sample is immobilized onto a stationary phase. For example, the

antigen is coated on the stationary phase such as a microplate. Preferably, the blood serum sample is diluted with a buffer solution before the immobilization with the dilution ratio of 32-256. After immobilizing the antigen, the stationary phase can be washed and blocked.

Then a monoclonal antibody (the 1st antibody of the galectin-3, for example, M3/38 monoclonal antibody (rat IgG)) is added to the stationary phase to react with the antigen, and washed, and then the 2nd antibody (for example, a horse radish peroxidase (HRP)-conjugated goat anti-rat IgG) is added to react with the 1st antibody which is also an antigen of the 2nd antibody. Preferably, the 2nd antibody is marked with enzyme. After the 2nd antibody marked with enzyme is combined to the 1st antibody, a colorant solution is added to the 2nd antibody to induce enzyme reaction and clear color change.

After stopping the enzyme reaction, the light absorbance of the gallectin-3 and the monoclonal antibody complex is measured to determine the concentration of the galectin-3 in the blood sample. Since the 1st antibody, the 2nd antibody, enzyme, and the colorant are generally well known in the art, the selection thereof can be easily carried out by skilled person in the art, and preferably only the 1st antibody marked with enzyme can be used without the 2nd antibody..

Alternatively, a kit such as an Immunochromatographic assay strip, which has similar configuration with the conventional kits for detecting pregnancy, diabetes or cholesterol content in blood, can be used for determining the concentration of the galectin-3.

Such kit may have various configurations which are well known in the art.

As shown in Fig. 6, the simplest configuration of the kit is an assay strip 100 which includes a reaction part 10, a sample injection part 20, and a membrane 30 for providing passage from the sample injection part 20 to the reaction part 10. The assay strip 100 can be protected by a plastic case having at least one window for observing the reaction part 10 and for injecting the sample.

In one embodiment, a capture antibody of the galectin-3 is immobilized to the reaction part 10, and a blood sample including the antigen (galectin-3) and gold-conjugated tracer antibody of the galectin-3 are moved trough the membrane 30 from the sample injection part 20 to the reaction part 10. In this embodiment if the galectin-3 is over-manifested in the blood sample, the more galectin-3 reacts with the gold-conjugated tracer antibody, and the greater amount of the antibody–antigen complex is captured on the reaction part 10, which results in the color change of the reaction part 10. On the contrary, if the galectin-3 is not over-manifested in the blood sample, the gold-conjugated tracer antibody cannot react with the antigen, thus cannot be captured on the reaction part 10. In this case, the color of the reaction part 10 cannot be changed. Therefore, a user can determine the concentration of the galectin-3 according to the color change of the reaction part 10.

The gold-conjugated tracer antibody can be directly mixed with the blood sample and injected onto the sample injection part 20. Alternatively, the gold-conjugated tracer antibody can be positioned between the sample injection part 20 and the reaction part 10 in a dried state. In latter case, the dried gold-conjugated tracer antibody melts when the blood sample moves from the sample injection part 20 to the reaction part 10, and reacts with the galectin-3 in the blood sample.

In other embodiment, galectin-3 is immobilized to the reaction part 10, and a blood sample including galectin-3 and gold-conjugated tracer antibody of the galectin-3 are moved trough the membrane 30 from the sample injection part 20 to the reaction part 10. When the blood sample including the galectin-3 and gold-conjugated tracer antibody reaches the reaction part 10, the galectin-3 immobilized onto the reaction part 10 and the galectin-3 in the blood sample competitively react with the gold-conjugated tracer antibody. Therefore, if the galectin-3 is over-manifested in the blood sample, the galectin-3 immobilized onto the reaction part 10 cannot react with the gold-conjugated tracer antibody. Thus, the color of the reaction part 10 cannot be changed. On the contrary, if the galectin-3 is not over-manifested in the blood sample, the galectin-3 immobilized onto the reaction part 10 captures large amount of the gold-conjugated tracer antibody, which results in the color change of the reaction part 10.

In summary, the reaction part 10 includes the capture antibody of galectin-3 or galectin-3 immobilized thereon so that the color of the reaction part 10 is determined according to the concentration of galectin-3 in the blood sample when the blood sample including galectin-3 and the gold-conjugated tracer antibody of the galectin-3 reaches to the reaction part 10 trough the membrane 30.

To clearly detect the color change of the reaction part 10, the blood sample can be decolorized according to the conventional method, such as mixing the blood sample with an oxidant. Alternatively, a filter for filtering the coloring matter in the blood sample can be formed between the sample injection part 20

and the reaction part 10. Preferably, a conventional control line 40 can be formed on the membrane 30 at the opposite end of the sample injection part 20, and the control line 40 is designed to change its color when the blood sample arrives to the control line 40, which makes the user to confirm the test is completed.

Generally, even the normal tissue includes small amount of galectin-3. Therefore, the color change of the kit should be controlled so that the color change should occur when the concentration of the galectin-3 in the blood sample is greater than that in the normal blood. In addition, the color change due to the concentration of the galectin-3 depends on the dilution ratio of the sample (See following Example). Therefore, the sample dilution ratio should be controlled so that the color of the reaction part cannot be changed with the normal blood, and can be changed with the abnormal blood. The dilution ratio can be properly controlled according to the kit configuration. In addition, any conventional kit configuration disclosed in U.S. Pat No. 5,616,467 can be used as a kit configuration of the present invention.

In order to more fully illustrate the preferred embodiments of the present invention, the following detailed examples are given.

Example

1. Material and method.

(1). Preparation of stomach cancer tissue and blood

100 stomach cancer tissue slides were obtained from the Aju University

Hospital in Republic of Korea. The tissue slides were collected during the gastrectomy operations of the 100 patients who were diagnosed to have stomach cancer by pathological diagnosis during 1994-1996. The tissue slides consisted of tissues under advanced carcinomas, early gastric carcinomas (EGC), precancerous lesions, and normal gastric mucosal tissues. The normal peripheral blood were obtained from normal person who are selected in random, and the peripheral blood of patients having stomach cancer or gastritis were obtained from the Aju University Hospital and Catholic University Vincent Hospital in Republic of Korea.

(2). Immunohistochemical staining of the galectin-3 protein in stomach cancer tissue

In order to remove the paraffin in the prepared slides and to hydrate the slides, the tissue slides were dipped into xylene solution for 5 minutes, and the dipping process was repeated 3 times. Then the slides were successively dipped into 100% ethanol solution, other 100% ethanol solution, 90% ethanol solution, 80% ethanol solution, and 70% ethanol solution each for 5 minutes. Then the slides were dipped into distilled water for 10 minutes, and 3% hydrogen peroxide solution for 5 minutes, and washed with PBS containing 1% Triton X-100, 3 times each for 5minutes. The washed slides were moved into a humidified chamber in order to prevent non-specific binding reactions, and treated with 10% normal goat serum, and reacted for 30minutes at room temperature.

After the reaction was complete, the culture supernatant of cell strain

producing M3/38 monoclonal antibody (rat IgG, 1st antibody) was added to the humidified chamber, and maintained at 4°C for over-night, and washed with PBS containing 1% Triton X-100 3 times each for 5minutes. As the 2nd antibody, a horse radish peroxidase (HRP)-conjugated goat anti-rat IgG (Pierce co.) was diluted with PBS containing 3% BSA with the dilution ratio of 1: 800, and was added to the humidified chamber, and reacted for 1 hour at room temperature, and washed with PBS containing 1% Triton X-100 3 times each for 5minutes. Then the tissue is reacted with 3,3'-diaminobenzidine (DAB), and washed, and stained with Harris' hematoxylin as control, and dehydrated.

(3). Measurement of concentration of the galectin-3 in peripheral blood

The concentration of the galectin-3 in peripheral blood was measured as follows with ELISA (Enzyme-linked Immunosorbent Assay).

100μl of buffer solution including same amount of 0.5M carbonate and 0.5M bicarbonate (pH = 9.6) was coated on two 96 well plates, respectively. Then, 50μl of the buffer solution and 50μl of the blood sample including antigen (galectin-3) was coated onto one of the 96 well plates. The mixture was mixed well, and 100μl of the mixture was transferred to the 2nd 96-well plate to dilute the sample 2 times. By repeating the dilution process, the mixture was diluted to 2048 times, and in some experiment, diluted to 131072 times.

The diluted mixtures were maintained at 4°C for over-night to coat the antigen(galectin-3) onto the 96-well plate. Then the coated antigen was washed with washing solution (PBS+ 0.05% Tween-20) 3 times, and blocked with PSB containing 3% BSA, and cultured for 1 hour at 37°C. Then the microplate was

washed.

Then, the 1st antigen was added to the coated antigen. In detail, the prepared monoclonal antibody of the galectin-3 was diluted with EIA buffer solution having the following composition with the dilution ratio of 1:1, and inoculated to the well(100 μl/well), and incubated for 2 hours at 37°C, and washed.

< Composition of EIA buffer solution (100ml) >

0.01M tris-HCl buffer solution	75ml
Tween-20	0.1ml
Fetal bovine serum	25ml
EDTA	200mg
Thimerosal	5mg

Then the 2nd antibody was reacted. In detail, goat anti rat Ig G-HRP was diluted with the dilution ratio of 1:1,500, and inoculated to the well (100 μl/well), and incubated for 2 hours at 37°C, and washed. The next step was to inoculate chromogen solution to the well (100 μl/well). At this time, a stock solution was prepared so that the final concentration of OPD was 0.5 ㎎/㎖, and the buffer solution of the OPD substrate consisted of 0.05M sodium citrate and 0.05M Na_2HPO_4 (pH 5.0). The HRP substrate was prepared by mixing 1 ㎖ of OPD stock (ortho-phenylenediamine stock solution), 9 ㎖ of OPD buffer solution, and 3.3 ㎕ of 30% H_2O_2, and the mixture was incubated for 1 hour at room temperature. Then the reaction was stopped by inoculation of 2.5M H_2SO_4 (50 ㎕ /well), and the light absorbance of the plate at 490 nm was measured.

(4). Pathological interpretation

The manifestation of galectin-3 at Auerbach plexus disposed in stomach was regarded as control. The pathological diagnosis was interpreted according to the following criteria.

(-): galectin-3 is not manifested

(1+): galectin-3 is manifested in the tumor cell of less than 5%

(2+): galectin-3 is manifested in the tumor cell of 5-50%

(3+): galectin-3 is manifested in the tumor cell of more than 50%

(5). Grouping and statistical analysis

The four criteria are divided into 2 groups as follows for comparing the pathological parameters according to the manifestation of the galectin-3.

A group: 3+

B group: -, 1+, 2+

Pathological parameters, such as a depth of invasion, number of lymph node metastasis, and TNM stage according to the UICC classification method between the two groups were analyzed with the Chi-square test method, and the ratios of survival of the two groups according to the manifestation of the galectin-3 was determined with Kaplan-Meier method. When P value was less than 0.05, the data is regarded as statistically useful.

2. Result

(1). Immunohistochemical staining

From the manifestation of the galectin-3 at Auerbach plexus (See Figs. 1A and 1B), it is clear that galectin-3 was seldom manifested in normal cell, and only small amount of the galectin-3 was manifested at the mucosal neck portion. As shown in Figs. 2A and 2B, the galectin-3 is more strongly manifested in the stage of intestinal metaplasia and adenoma which are former stages of the tumor. As shown in Fig. 3, the galectin-3 is widely manifested in the tumor tissue, but the degree of the manifestation increases with the degree of differentiation of a cell. Thus the degree of the manifestation at the stage of the differentiation of a cell is greater than that of the stage of the malignant tumor.

(2). Comparison of the two groups according to the degree of manifestation of the galectin-3

100 patients having stomach cancer included 72 men and 28 women, and their average age was 53.7 (age range: 27-78). Among them, 64 people were classified into the group A, and 36 people were classified into group B. The pathological parameters of the two groups were compared, and the results are shown in Table 1. In case of group B, the numbers of intestinal type and diffuse type are almost same, but in case of group A, the number of intestinal type was greater than that of the diffuse type ($p<0.05$). In case of the depth of invasion, the ratio of T3 was the greatest in both of the two groups ($p<0.05$), and the number of lymph node metastasis was in the range of 1-6($p<0.05$).

The ratios of stage III among stage I-IV were greatest, but the p-value was

greater than 0.05, and the test results were regarded as not useful statistically.

Table 1: Pathological analysis of the patient groups according to the manifestation of the galactin-3.

Pathological parameter	Group A Number(%)	Group B Number(%)	P-value
Lauren			
Intestinal Type	52(81.3)	17(47.2)	< 0.05
Diffuse Type	12(18.8)	19(52.8)	
Depth of Invasion			
T1	8(12.5)	3(8.3)	< 0.05
T2	6(9.4)	7(19.4)	
T3	50(78.1)	23(63.9)	
T4	0(0)	3(8.3)	
No. of Positive LNs			
0	17(26.6)	3(8.3)	< 0.05
1-6	22(34.4)	20(55.6)	
7-15	14(21.9)	10(27.8)	
more than 16	11(17.2)	3(8.3)	
Stage			
I	8(12.5)	3(8.3)	0.648
II	16(25.0)	6(16.7)	
III	29(45.3)	20(55.6)	
IV	11(17.2)	7(19.4)	

The degree of manifestation of galectin-3 of Group A(n=64): 3+

The degree of manifestation of galectin-3 in Group B(n=36): -,1+, 2+

As shown in Fig.4 showing the ratio of 5 years survival according to the manifestation of the galectin-3, the ratio of 5 years survival of the Group A was 64.06%, and the ratio of 5 years survival of the Group B was 63.89% (p value = 0.9153). Thus, it is found that the manifestation of the galectin-3 has no relation with the expected life time of the patients.

(3). Detection of the galectin-3 at peripheral blood.

The peripheral blood was obtained from the groups of normal human, patient having chronic gastritis, patient having benign tumor, and patient having stomach cancer, respectively. The concentrations of galectin-3 were measured, and the results are depicted in Fig. 5. As shown in Fig.5, the manifestations of the galectin-3 of the groups of chronic gastritis, benign tumor, and stomach cancer were greater than that of normal human. Thus, the galectin-3 can be used more effectively as a cancer anticipating or predicting antigen rather than cancer diagnosing agent. The discriminating power of the manifestations was maximized with 100 times dilution of the blood sample.

3. Discussion

From the above-described experiment, the degree of manifestation of the galectin-3 at each stage of the tumor development was determined, and its relation with the tumor developments was discovered. In sum, the galectin-3 seldom manifests in normal cell, but strongly manifests in the stage of intestinal metaplasia and adenoma which are the former stages of the tumor. The most unexpected result obtained from the experiment is that the galectin-3 more strongly manifests in the stage of intestinal metaplasia and adenoma than in the stage of the tumor. The manifestation of the galectin-3 is more stronger in the well differentiated tumor cell than in undifferentiated tumor cell. This means that the manifestation of the galectin-3 increases with the initiation of intestinal metaplasia and adenoma, and is maximized during the stages of intestinal

metaplasia and adenoma, and maintained when the intestinal metaplasia and adenoma are developed to tumor.

From the pathological parameter analysis, the manifestation of the galectin-3 had little relation with the depth of invasion and the number of lymph node metastasis. This result is similar to the result of research of breast carcinoma, and different from the result of research of colon cancer, lung cancer, melanoma etc, in which the manifestation of the galectin-3 has close relation with development of malignant tumor. From the experiment to the stomach cancer, it is found that the manifestation of the galectin-3 has little relation with the ratio of survival of patients (p=0.9153), which means that the galectin-3 is not so effective as a diagnostic marker specific to the malignant tumor.

However, in the present invention, the galectin-3 is mainly used as a diagnosing agent of the intestinal metaplasia and adenoma rather than the malignant tumor. The galectin-3 is expected to have a role in the initial or middle stages of the development of carcinogenesis since the galectin-3 has mainly manifested at the former or initial stage of the development of the stomach cancer. Therefore, the manifestation of the galectin-3 is useful as a screening marker for early predicting of stomach cancer and the probability of contracting stomach cancer.

4. Conclusion

It is reported that the manifestation of the galectin-3 increases in the malignant tumor such as colon cancer, thyroid gland cancer, lung cancer and

black epithelioma, and decreases in the breast cancer. In case of stomach cancer, it is reported that the galectin-3 seldom manifest in the normal tissue of stomach, but manifest in tissue of the stomach cancer [30]. In the above report, the manifestation of the galectin-3 in normal stomach tissue and stomach cancer was studied, but the manifestation of the galectin-3 during development of cancer was not established, thus the use of galectin-3 as an antigen for early predicting of tumor was not developed in the prior report. In contrast, the manifestations of the galectin-3 in normal tissue and blood, former stage of stomach cancer and initial stage of benign tumor and complicated malignant tumor were studied in the present invention, and it is found that the over-manifestations of the galectin-3 can be used as a signal for initiation of the malignant tumor, namely, a signal for detecting the former and initial stages of the tumor development.

In this disclosure, there is shown and described only the preferred examples of the invention, but, as aforementioned, it is to be understood that the invention is capable of use in various other combinations and environments and is capable of changes or modifications within the scope of the inventive concepts as expressed herein.

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CLAIMS

1. A method of diagnosing or predicting the occurrence of a cancer or the risk of contracting a cancer comprising the steps of:

determining a concentration of galectin-3 in a blood sample by reacting the blood sample with a monoclonal antibody of the galectin-3;

comparing the determined concentration of the galectin-3 with concentration of the galectin-3 in a blood sample of a normal human; and

predicting the risk of contracting a cancer if the determined concentration is greater than the concentration of the galectin-3 in blood of the normal human.

2. The method according to claim 1, wherein the step of determining the concentration of galectin-3 in the blood sample is carried out by measuring absorbance of the gallectin-3 and the monoclonal antibody complex, which is formed by enzyme-linked Immunosorbent assay (ELISA) method.

3. The method according to claim 2, wherein the monoclonal antibody, which is a first antibody, is M3/38 monoclonal antibody (rat IgG), and a horse radish peroxidase (HRP)-conjugated goat anti-rat IgG is used as a second antibody which reacts with the first antibody.

4. The method according to claim 1, wherein the step of determining the concentration of galectin-3 in the blood sample comprises the steps of:

diluting the blood sample with a buffer solution to a predetermined dilution ratio;

fixing the galectins-3 in the blood sample on a stationary phase;

reacting the monoclonal antibody of the galectin-3 as the first antibody with the galectins-3 fixed on the stationary phase;

reacting a horse radish peroxidase (HRP)-conjugated goat anti-rat IgG as the second antibody with the first antibody;

applying a colorant solution to the horse radish peroxidase; and

determining the concentration of the galectins-3 by measuring an absorbance of the galectin-3 and the antibody complex.

5. The method according to claim 1, wherein the concentration of galectin-3 in the blood sample is determined while diluting an original blood sample with a dilution ratio of from 32 to 256.

6. A method of diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising the steps of:

forming an assay strip having a reaction part, a sample injection part, and a membrane for providing passage from the sample injection part to the reaction part, wherein a capture antibody of galectin-3 or galectin-3 is immobilized on the reaction part ;

moving a blood sample including galectin-3 and a gold-conjugated tracer antibody of the galectin-3 trough the membrane from the sample injection part to the reaction part; and

predicting the risk of contracting a cancer according to the color change of the reaction part.

7. The method according to claim 6, wherein the gold-conjugated tracer antibody is directly mixed with the blood sample and injected onto the sample injection part.

8. The method according to claim 6, wherein the assay strip further includes the gold-conjugated tracer antibody positioned between the sample

injection part and the reaction part in a dried state.

9. The method according to claim 6, wherein the assay strip further includes a control line formed on the membrane at the opposite end of the sample injection part, which is designed to change its color when the blood sample arrives to the control line.

10. The method according to claim 6, wherein the blood sample is diluted with a dilution ratio of from 32 to 256.

11. A kit for diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising: an assay strip having a reaction part; a sample injection part: and a membrane for providing sample passage from the sample injection part to the reaction part,

wherein the reaction part includes a capture antibody of galectin-3 or galectin-3 immobilized thereon so that a color of the reaction part is determined according to the concentration of galectin-3 in a blood sample when the blood sample including galectin-3 and a gold-conjugated tracer antibody of the galectin-3 reaches from the sample injection part to the reaction part trough the membrane.

12. A kit according to claim 11, wherein the gold-conjugated tracer antibody is positioned on the assay strip between the sample injection part and the reaction part in a dried state.

13. A kit according to claim 11, wherein the assay strip further includes a control line formed on the membrane at the opposite end of the sample injection part, which is designed to change its color when the blood sample arrives to the control line.

14. A kit for diagnosing or predicting the occurrence of cancer or the

risk of contracting a cancer comprising:

a microplate for immobilizing galectin-3 in a blood sample; and

a monoclonal antibody to react with the galectin-3 immobilized on the microplate to induce a color change of the microplate.

15. A method of diagnosing or predicting the occurrence of cancer or the risk of contracting a cancer comprising the steps of:

determining a cancer screening antigen which manifests in the stage of a adenoma or a chronic inflammation which are the former stages of the malignant tumor development;

determining a concentration of the cancer screening antigen in a blood sample by reacting the blood sample with a monoclonal antibody of the cancer screening antigen;

comparing the determined concentration of the cancer screening antigen with a concentration of the cancer screening antigen in a blood sample of a normal human; and

predicting the risk of contracting a cancer if the determined concentration is substantially greater or lesser than the concentration of the cancer screening antigen in blood of the normal human.

16. A method according to claim 16, wherein the step of determining a cancer screening antigen comprises the steps of:

measuring the degree of the manifestation of an antigen in tissue at the stage of normal, chronic inflammation, adenoma, and malignant tumor; and

determining the antigen as the cancer screening antigen when the degree of the manifestation of the antigen in tissue at the stage of chronic inflammation or

adenoma is substantially greater or lesser than the degree of the manifestation of the antigen in tissue at the stage of normal.

DRAWINGS

FIG. 1a

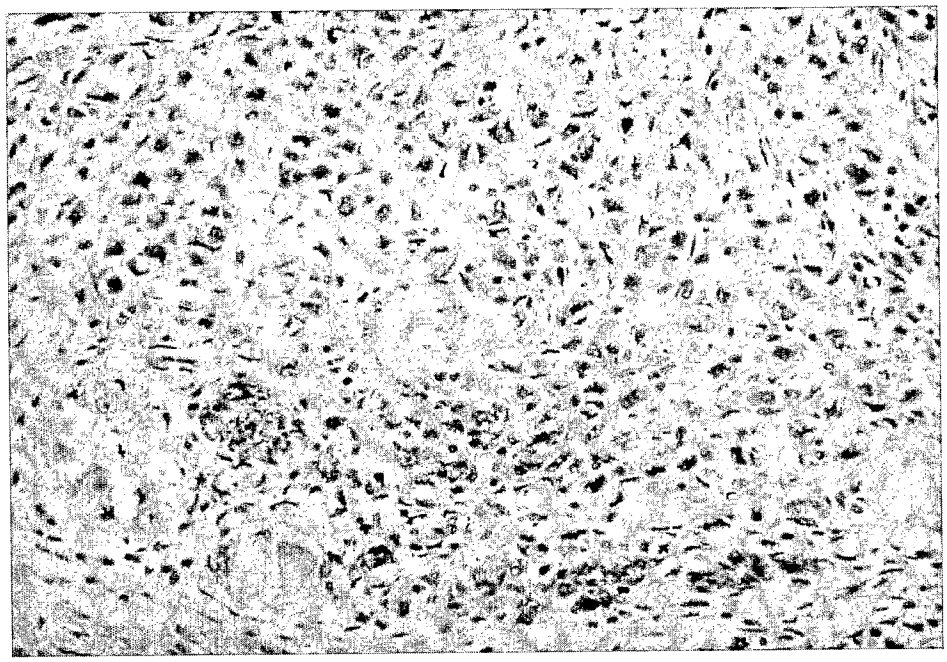


FIG. 1b

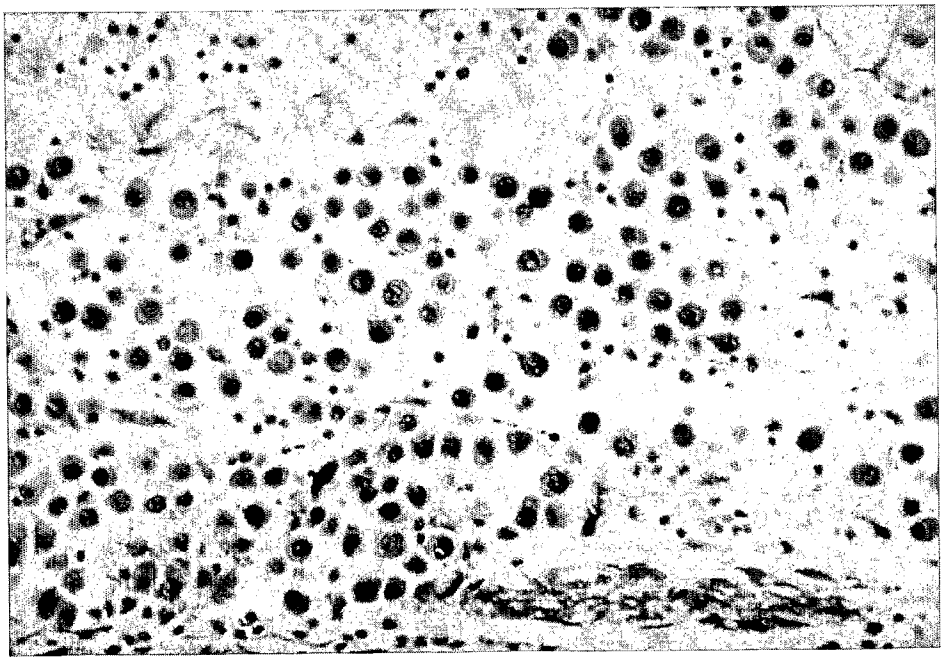


FIG. 2a

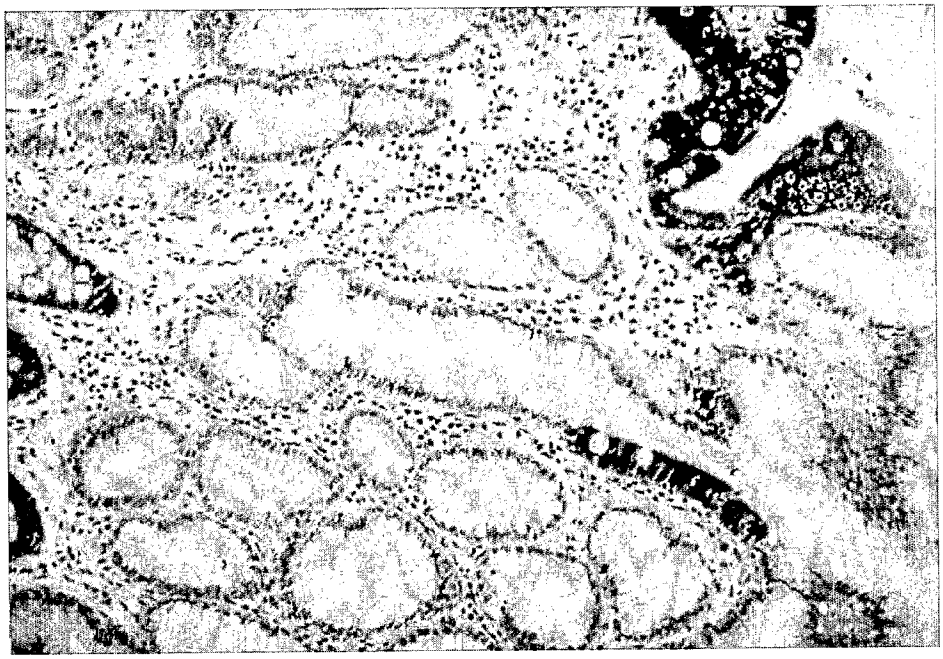


FIG. 2b

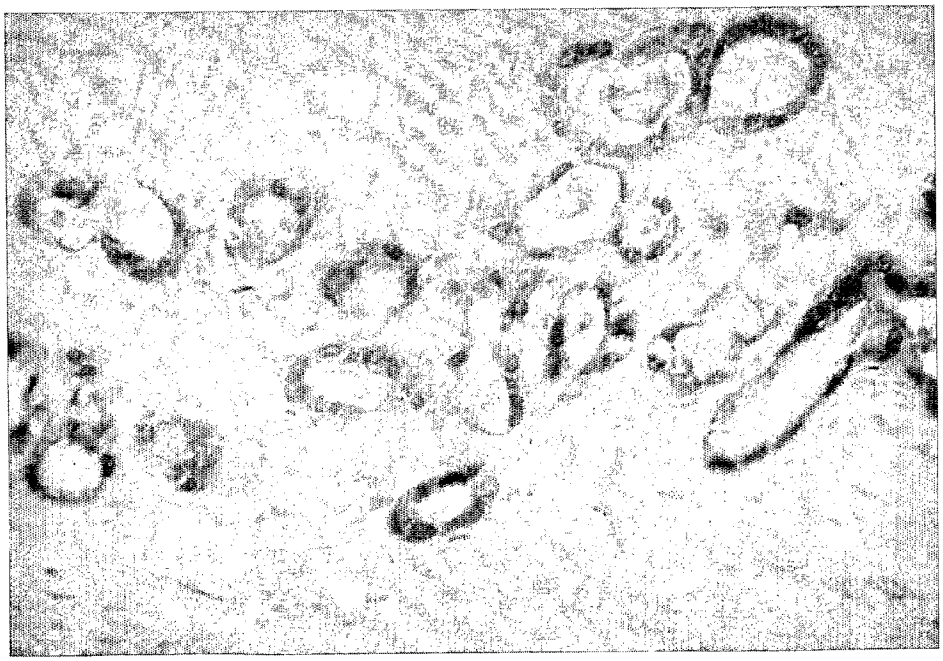


FIG. 3

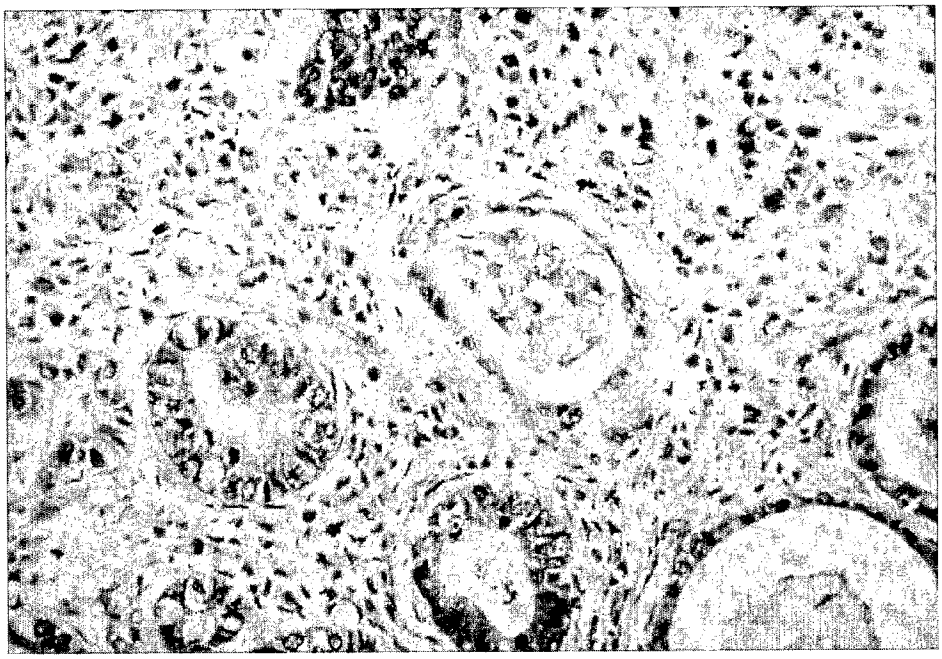


FIG. 4

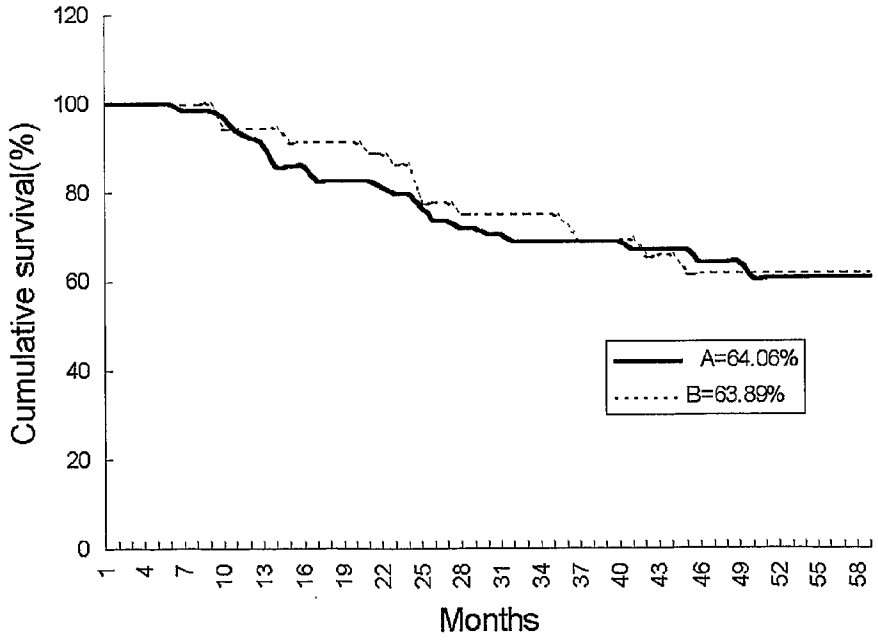
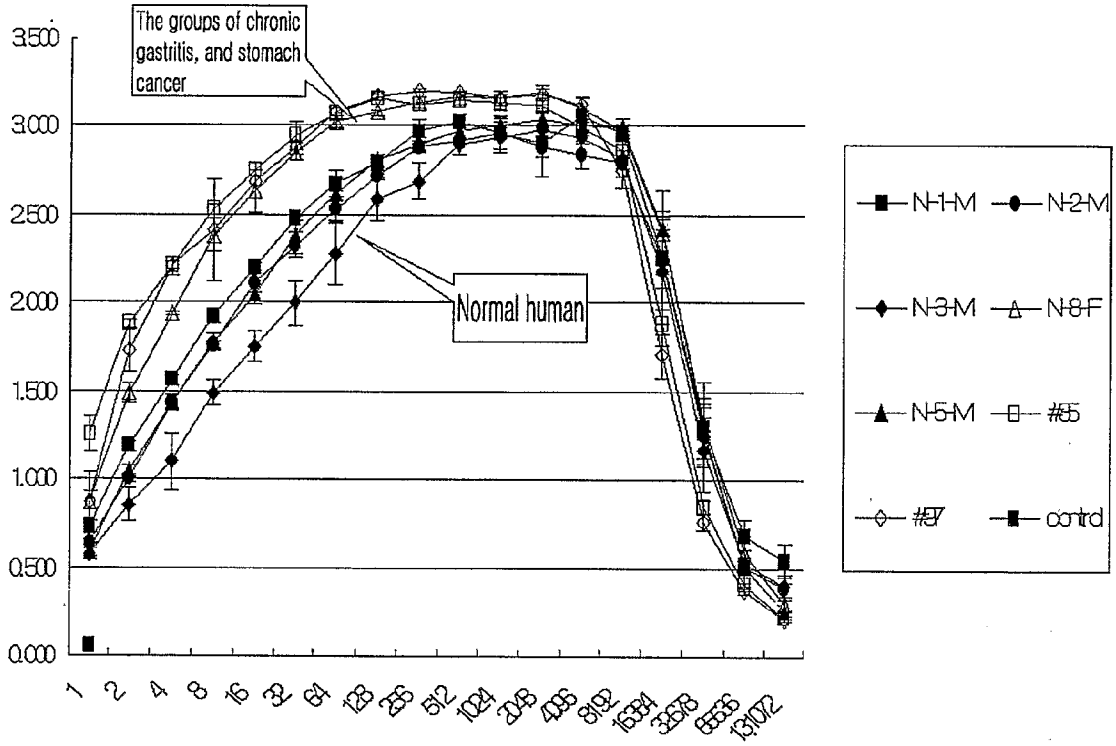

120
100
80
60
40
20
0
Cumulative survival(%)
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
Months
A=64.06%
B=63.89%

FIG. 5


The groups of chronic gastritis, and stomach cancer
Normal human
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
1
2
4
8
16
32
64
128
256
512
1024
2048
4096
8192
16384
32768
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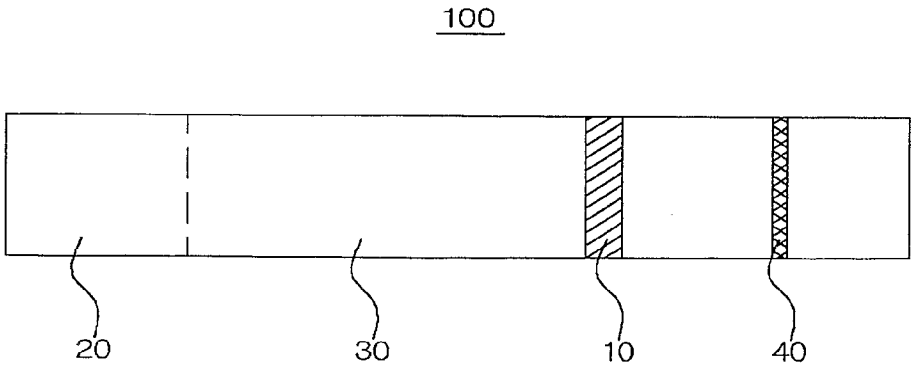
#57
contrd

FIG. 6


100
20
30
10
40

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/KR02/00249

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 G01N 33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 GO1N 33/574, A61K 31/715, C12N 15/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean patents and applications for inventions since 1975, Korean Utility models and applications for Utility models since 1975
Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

KIPASS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5, 801,002 A (Barbara Ann Karmanos Cancer, Inc) Sep. 1, 1998 - see the whole document -	1-16
A	US 5, 837,493 A (Incyte Pharmaceuticals, Inc) Nov. 17, 1998 - see the whole document -	1-16
A	US 5,869,289 A (Incyte Pharmaceuticals, Inc) Feb. 9, 1999 - see the whole document -	1-16
A	US 6,146,849 A (University of Georgia Research Foundation, Inc) Nov. 14, 2000 - see the whole document -	1-16
A	US 6, 281,333 B1(Incyte Genomics, Inc) Aug.28, 2001 - see the whole document -	1-16

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 NOVEMBER 2002 (20.11.2002)	20 NOVEMBER 2002 (20.11.2002)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	JOO, Young Sik Telephone No. 82-42-481-5995

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00249

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-10, 15-16
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 1-10, 15-16 relate to diagnostic methods of cancer, the search has been carried out and based on the alleged effects of the reagent and kit..

2. [ ] Claims Nos.:
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. [ ] As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.